The total hip arthroplasty (THA) provides an effective treatment for, for instance, osteoarthritis of the hip. Unfortunately, after 15-20 years, approximately 5-10% of all primary THAs fail, with "aseptic" loosening and osteolysis as the main reason. Various theories, in which the local inflammatory reaction is recognized, on the loosening process have been proposed, but have been unable to completely explain the mechanism. Even though the loosening process is thought to be sterile, several studies indicate the presence and an adverse role of microorganisms.
This thesis focused on various clinical aspects of the loosening problem and studied the local inflammatory environment and microorganisms in the loosening process. **(Chapter 1)**

The long term "aseptic" loosening mechanism has multiple aspects, both clinical and biological. The local inflammatory environment and possible adverse role for microorganisms are reviewed in **Chapter 2**. Two complementary mechanisms are acknowledged in efforts to unravel the loosening process, wear particles and high local pressure. Wear particles are released over time from the implant materials and migrate together with joint fluid between the bone and implant where they contribute to the local inflammatory response. Mechanical forces, lack of osseointegration, early implant migration and migrated joint fluid account for the high local pressure, which can also cause inflammation and subsequent osteolysis and loosening. The local inflammatory environment surrounding loosened hip implants has been studied extensively. Interface tissue retrieval studies revealed that macrophages represent the key cell type in these tissues and it was suggested that they play a key role in osteolysis and loosening. Also, cytokine profiles have been reviewed and several pro inflammatory cytokines, such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)α, were identified. These cytokines are capable of inducing bone resorption and are secreted by a.o. local macrophages. The macrophages could be attracted and activated by wear particles or high local pressure. The exact means by which the bone resorption is induced, is not fully understood. The pro-osteolytic factors receptor activator of nuclear factor-kappaB (RANK), RANK ligand (RANKL) and anti-osteolytic osteoprotegrin (OPG) are part of this process, since relatively high amounts of RANK and relatively low amounts of OPG are found in the interface tissues. Pro-inflammatory factors such as TNFα

and IL-1 have been shown to stimulate RANK, thus inducing bone resorption. Another way by which bone resorption could be enhanced is that the local macrophages are in fact osteoclast progenitor cells, which fuse and mature into the bone resorbing osteoclasts. Macrophages also fuse into foreign body giant cells, which form an important part of the local inflammatory foreign body reaction. Even though wear particles and high local pressure can account for a great part of the mechanism, both concepts are unable to fully explain the loosening process. In further analyses in solving this problem, various reports indicated a role for microorganisms. In a simplified hypothetical scheme, their possible role was proposed in the complexity of the local tissue response in long term hip implant loosening.

Apart from the local loosening process, long term hip implant loosening has great clinical implications for the patient, such as removal and replacement of the prosthesis. To understand the clinical degree of loosening and infection, a retrospective study was conducted (**Chapter 3),** in which population demographics and various factors were studied to identify the clinical risk factors in relation to hip implant loosening and postoperative infection. Several risk factors that increase the infection risk and decrease implant survival time, were identified such as a high age at primary surgery, obesity, long operating time and revision surgery, which is consistent with other reports. Implant fixation with bone cement reduced the implant survival time. Rheumatoid arthritis did not seem to have an influence on either while diabetes mellitus showed a trend towards increasing infection risk. A new risk factor was also identified, namely dementia, which increases the risk of infection.
All these factors should be assessed in the approach to long term hip implant loosening and implant related infection.

Whether or not microorganisms are present in "aseptically" loosened hip implants was a matter of debate. To clarify this problem, a more sensitive method for the detection of bacteria was developed and executed on interface tissues retrieved from patients with an "aseptically" loosened hip implant (**Chapter 4**). The universal primers for the detection of bacterial DNA, the 16S rRNA gene, were

used for quantitative PCR. After establishing sensitivity levels and validation of the method, it became apparent that the interface tissues of 37.5% of all included patients diagnosed with an "aseptically" loosened hip contained bacterial DNA. This means that implant related infections are currently underestimated. However, the direct clinical consequence of this finding is unclear. To better understand the local micro-environment, clinical interface tissue histology was studied in conjunction with the newly identified infected patients. The histological observations did not predict infection since no differences were found between interface tissues from "aseptic" loosenings that were either found to contain or to be devoid of bacterial DNA with our improved quantitative 16S rRNA PCR method. Another notable observation was great tissue heterogeneity was found within and between the interface tissues. Macrophages and/or foreign body giant cells, however, were always present in each tissue sample and often showed phagocytosed wear particles, indicating that these represent the key regulatory cell type in loosening. No differences in local inflammatory response were identified between 16S rRNA positive and negative samples.

The above mentioned study demonstrated that bacteria are present and that macrophages probably represent the key cell in the "aseptic" loosening process. The role of macrophages in relation to a biomaterial and microorganisms was further determined in an *in vivo* implant infection model (**Chapter 5).** In this model, a biomaterial was implanted subcutaneously and infected with *S. aureus*. Prior to the implantation, circulating macrophages were depleted by clodronate containing liposomes. Interestingly, bacterial infection was resolved in the depleted group while in the non-depleted group, infection was not cleared after day 28 and repeatedly exacerbated. In the non-depleted group, the macrophages seemed to mainly direct their action towards the degradable biomaterial or towards the bacteria, eventually resulting in a sustained local infection. This indicated that macrophages have a key role in a biomaterial related infection. Since bacteria seem to be involved in "aseptic" loosening, this finding provides further insight in the loosening process.

Apart from the role of macrophages, the role of bacteria in "aseptic" loosening was studied as well. A novel *in vivo* model was successfully developed, in which the end stages of loosening were simulated (**chapter 6**). An *S. epidermidis* biofilm contaminated titanium rod was inserted into a rat femur together with polyethylene particles. Radiographs of the femurs revealed bone pathological events from day 14 onward in all groups except the group containing only the titanium implant. After 56 days, the experimental group displayed the most distinct bone pathological consequences.

Histologically, the local inflammatory response from the experimental group resembled the response found in the interface tissues retrieved from patients undergoing THA revision surgery for "aseptic" loosening, suggesting bacterial involvement in the loosening process. Also, in the two groups in which a bacterial biofilm was inserted, *S epidermidis* was not evenly distributed throughout the bone-implant interface tissue. Also, after explantation, several rods were bacterial culture negative after sonication while bacteria were found in the surroundings, supporting the results from the interface retrieval study (chapter 4) which demonstrated that not all interface tissue samples were positive within one patient.

Apart from the local response, the systemic response in both rats and patients was studied as well. The rat cytokine profiles at day 14 in the groups containing microorganisms mostly resembled the cytokine profiles found in patients with an "aseptically" loosened hip implant, while the groups without microorganisms did not.

All this demonstrates that bacteria probably have a role in the loosening process.

In conclusion, clinical parameters, the local environment and microorganisms have a significant part in "aseptic" loosening of hip prostheses. A multifactorial approach can help resolve the problem. More knowledge on the local environment and the role of microorganisms can lead to novel intervention and modulation methods, which will result in inhibiting or preventing the local long term loosening process.